Comparisons of lactated Ringer's and Hextend resuscitation on hemodynamics and coagulation following femur injury and severe hemorrhage in pigs

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BACKGROUND: This study compared coagulation function after resuscitation with Hextend and lactated Ringer's (LR) solution in pigs with tissue injury and hemorrhagic shock.

METHODS: Pigs were randomized into control (n = 7 each), LR, and Hextend groups. Femur fracture was induced using the captive bolt stunner at midshaft of the pigs' left legs, followed by hemorrhage of 60% total blood volume and resuscitation with either Hextend (equal to bled volume) or LR to reach the same mean arterial pressure. Pigs in the control group were not bled or resuscitated. Hemodynamics was monitored hourly for 6 hours. Blood samples were taken at baseline (BL), after hemorrhage, 15 minutes, 3 hours, and 6 hours after resuscitation for blood and coagulation measurements.

RESULTS: Mean arterial pressure decreased to 50% of BL by the 60% hemorrhage but returned to near BL within 1 hour after LR or Hextend resuscitation. Heart rate was increased (from 91 ± 4 beats per minute to 214 ± 20 beats per minute) by hemorrhage and decreased after resuscitation but remained elevated above BL in both groups. Resuscitation with Hextend (42 mL/kg) or LR (118 ± 3 mL/kg) reduced hematocrit, total protein, fibrinogen, and platelet counts, with greater decreases shown in the Hextend group. Clot strength was lower but returned to BL by 3 hours in the LR group, whereas it remained reduced for the 6-hour period after Hextend. The overall clotting capacity after LR was decreased after hemorrhage and resuscitation but returned to BL by 3 hours, whereas it remained low after Hextend for the 6-hour experiment period.

CONCLUSION: After traumatic hemorrhage, coagulation function was restored within 6 hours with LR resuscitation but not with Hextend. The lack of recovery after Hextend is likely caused by greater hemodilution and possible effects of starches on coagulation substrates and further documents the need to restrict the use of high-molecular-weight starch in resuscitation fluids for bleeding casualties. (*J Trauma Acute Care Surg*. 2013;74: 732–740. Copyright © 2013 by Lippincott Williams & Wilkins)

KEY WORDS: Hemorrhagic shock; Hextend; lactated Ringer's (LR) solution; thrombelastography; pig.

Hemorrhage is the leading cause of potentially survivable death in the battlefield and a major cause of death in civilian trauma.[1] Blood loss is also commonly encountered during surgical intervention.[2] To restore tissue perfusion and hemodynamics, fluid resuscitation is a routine clinical practice to treat hypovolemia. A variety of resuscitation fluids have been used around the globe, with selections depending on availability, cost, and clinical experience.[3] Crystalloids, such as isotonic sodium chloride solution and lactated Ringer's (LR) solution, are inexpensive and have been widely used at prehospital and hospital settings. Colloids, such as albumin, gelatin, and hydroxyethyl starch (HES), are highly effective in increasing intravascular volume with small volume increase in interstitial space (compared with crystalloids). Different colloid products have been used in clinical practice.[4–7] As one of the HES products, Hextend (BioTime, Berkeley, CA) was developed and approved for use as a plasma volume expander in the United States in 1999 and was recommended for use in the military by the committee on Tactical Combat Casualty Care.[8] It contains 6% hetastarch with a mean molecular weight (MW) of 670 kD and a degree of hydroxyethyl group substitution of 0.75 (HES 670/0.75) in a physiologically balanced solution of electrolytes, glucose, and lactate. The newest starch product, Voluven (Fresenius Kabi, Bad Homburg, Germany), was developed and approved in Europe and recently in the United States and contains 6% Hetastarch with a MW of 130 kD and a degree of hydroxyethyl group substitution of 0.4 (HES 130/0.4) in saline medium. A growing number of studies have been undertaken to investigate resuscitative effects of colloids.[5,6,9–14] Although effective volume expansion and positive clinical outcomes have been reported in clinical trials and animal studies,[7,15–16] colloid infusion has been shown to be associated with reductions of coagulation factors,[17] platelet dysfunction,[18–20] anaphylactic reactions,[21] and hemorrhagic complications.[22–24]

Limited information is available comparing Hextend with crystalloid LR after hemorrhage in vivo. In a swine model, Todd et al.[25] investigated effects of LR and Hextend on coagulation changes and blood loss after severe liver injury. However, since the only coagulation data shown were at the end of the study,[25] it is difficult to evaluate the time course changes in coagulation from either LR or Hextend resuscitation or compare the effects between LR and Hextend from that study. In patients who

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underwent knee replacement surgery, Innerhofer et al.[9] compared resuscitative effects of Voluven with LR and showed greater reductions in final clot strength in patients resuscitated with Voluven compared with those with LR. It remains unknown whether the coagulation effects of starches vary with MW and substitution.

In this study, we investigated the effects of Hextend and LR on hemodynamics and coagulation in a swine model with traumatic hemorrhage. Following femur fracture and 60% hemorrhage, LR or Hextend was infused, and changes of hemodynamics, coagulation, and physiology were compared with prehemorrhage values and monitored for 6 hours after resuscitation.

MATERIALS AND METHODS

This study was approved by the Institutional Animal Care and Use Committee of the US Army Institute of Surgical Research and was conducted in compliance with the Animal Welfare Act and the Animal Welfare Regulations in accordance with the principles of the Guide for the Care and Use of Laboratory Animals.

A total of 21 crossbred Yorkshire swine (38.5 ± 0.9 kg) were randomized into three groups (n = 7 each) as follows: the sham control (control); hemorrhage with LR resuscitation (LR); and hemorrhage with Hextend resuscitation (Hextend). After an overnight fasting, the animals were preanesthetized with glycopyrrolate (0.1 mg/kg) and Telazol (6 mg/kg), intubated, and anesthetized with 1.0% to1.5% isoflurane in 100% oxygen by mask for the surgical procedures. Polyvinyl chloride catheters were inserted into the thoracic aorta via the carotid artery to measure mean arterial pressure (MAP), heart rate, and temperature. The right femoral artery was cannulated for arterial blood sampling, and the left femoral artery was cannulated for the hemorrhage procedure. The left femoral vein was cannulated for LR resuscitation. The right femoral vein was cannulated for intravenous anesthesia of ketamine during the study. No splenectomy was performed in this study.

Upon the completion of cannulation, animals (except for the control group) were subjected to femur fracture and hemorrhage while still being maintained under a surgical plane of anesthesia. Femur fracture was induced by a captive bolt fired at the animal's left thigh. Blood loss from the injury was determined with preweighed lap sponges. Afterward, hemorrhage of 60% estimated total blood volume (42 mL/kg) was induced by bleeding from the femoral artery with a computer-controlled pump into sterile empty blood bags containing standard anticoagulant. Each pig was exponentially bled to mimic reduced blood flow as blood pressure drops. Upon the completion of hemorrhage, animals underwent a "shock period" phase of 15 minutes, followed by randomization into Hextend group or LR group. In the Hextend group, a Hextend solution of equivalent bled volume (42 mL/kg) was given to the animals. LR solution was infused in the LR group to recover MAP to the same levels as in the Hextend group. After resuscitation, the animals were monitored for 6 hours. No shed blood was returned to the pigs.

Blood samples were taken before femur fracture (baseline), at 15 minutes after hemorrhage (hemorrhage time point), 15 minutes after resuscitation (resuscitation time point), 3 hours, and 6 hours after resuscitation for measurements of blood gas, blood chemistry, and coagulation. MAP, heart rate, and temperature were monitored hourly during the study. Cardiac output was measured at baseline and at 15 minutes, 3 hours, and 6 hours after hemorrhage and resuscitation. After blood sampling at 6 hours, the animals were euthanized with a sodium pentobarbital euthanasia solution (FatalPlus, Fort Dodge, IA) given intravenously.

Analytical Methods

Blood gas measurements were determined by the Omni-9 Blood Gas Analyzer (AVL, Montpellier, France). Blood chemistries were measured by the Dimension Clinical Chemistry System (Dade Behring, Newark, DE). Platelet counts were measured from citrated blood using an ABX Pentra 120 Hematology Analyzer (ABX Diagnostics, Inc., Irvine, CA). Plasma fibrinogen concentrations and coagulation factors were measured using the BCS Coagulation System (Dade Behring, Deerfield, IL).

The coagulation profile was assessed in fresh whole-blood samples with tissue factor using thrombelastography (TEG, TEG 5000 Hemostasis Analyzer, Haemoscope Corp, Niles, IL) as described previously.[26] TEG measurements included *R* time (the time that the initial clot is formed), *K* time (the time when clot strength reaches 20 mm), maximum amplitude (MA) (maximum clot strength), α angle (the rapidity of fibrin buildup and cross-linking), and LY_{60} (rate of amplitude reduction 60 minutes after MA to determine fibrinolysis). Coagulation index (CI) represents the overall coagulation status[6] and was calculated as $CI = -0.1227R + 0.0092K + 0.1655MA - 0.0241\alpha$. TEG measurements were made in triplicate at every time point and performed for 1.5 hours to ensure the completion of LY_{60}.

Statistical Analysis

Data were expressed as mean ± SEM and analyzed using SAS statistical software (Cary, NC). A two-way analysis of variance with repeated measures using a Tukey's adjustment was used to compare the changes over time between the groups. A one-way analysis of variance with repeated measures using a Dunnett's adjustment was used to compare the changes to baseline within the group. The statistically significant level was set at $p < 0.05$.

RESULTS

Hemodynamics and Blood Measurements

All of the animals from the 3 groups survived to the end of the study. All baseline measurements were similar among the groups. No significant changes were observed in any measurements in the control group during the study period.

MAP was similarly reduced by hemorrhage in the LR and Hextend groups and returned to baseline values by resuscitation with Hextend (42 mL/kg) or LR (118 ± 3 mL/kg) (Fig. 1). Heart rate was increased by hemorrhage in both groups (Fig. 1). Elevated heart rate was decreased by resuscitation with LR or Hextend but remained elevated above baseline for the 6-hour experimental period in both groups (Fig. 1). Cardiac output was increased after hemorrhage and resuscitation, returned to baseline at 3 hours with LR resuscitation, but remained elevated at

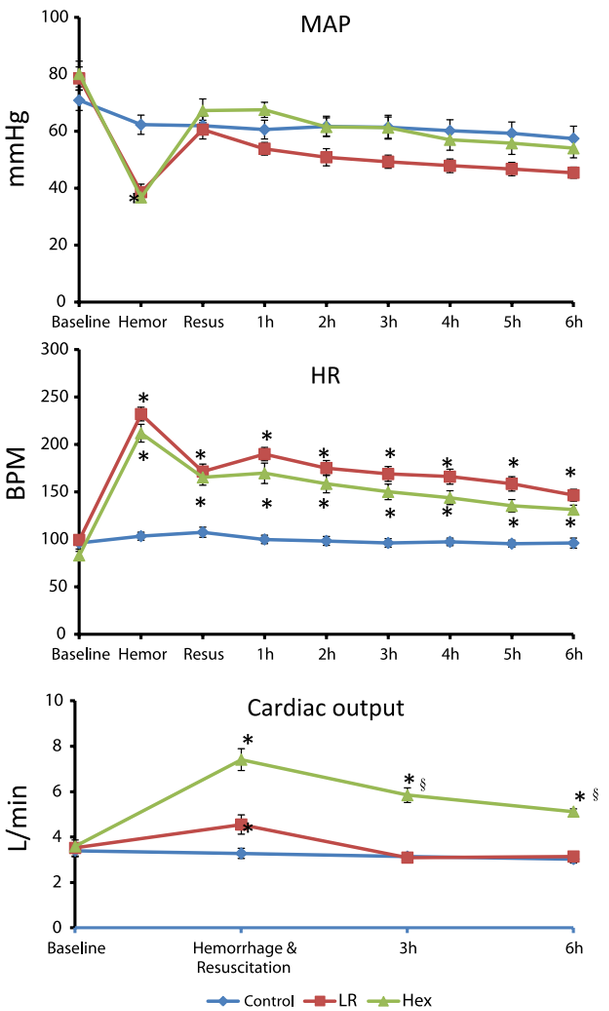

Figure 1. Changes of MAP, heart rate, and cardiac output after femur fracture, hemorrhage, and LR solution or Hextend resuscitation in pigs. Data are represented as mean ± SEM for 7 animals per group. *$p < 0.05$ compared with the corresponding baseline values. §$p < 0.05$ LR group compared with the Hextend group.

6 hours with Hextend resuscitation (Fig. 1). No significant changes in body temperature were observed in any animal group during the study (data not shown).

Lactate was similarly increased by hemorrhage in both hemorrhage groups (Table 1). Lactate returned to prehemorrhage levels by 6 hours after Hextend resuscitation but remained elevated after LR resuscitation (Table 1). Base excess was decreased by hemorrhage but improved to near prehemorrhage levels with Hextend resuscitation (Table 1). At 6 hours in the LR group, base excess was approximately half of that in the control and Hextend groups. Hematocrit was decreased by hemorrhage and resuscitation in both groups for 6 hours, with larger decreases shown in the Hextend group (Table 1). Similarly, greater hemodilutions of red blood cells, hemoglobin, and total protein were observed in the Hextend group during the 6-hour experimental period (Table 1).

Blood amylase level did not change after hemorrhage but reduced after resuscitation with LR or Hextend, with a greater reduction shown in the Hextend group (Table 1). Afterward, an increase of amylase level was observed in the Hextend group, with no changes occurred in the LR group during the 6-hour study period (Table 1).

Fibrinogen, Platelet, and Coagulation Factors

Changes in plasma fibrinogen concentrations and platelet counts during the study are shown in Figure 2. Fibrinogen concentration fell by hemorrhage and resuscitation, with greater reduction shown after Hextend resuscitation compared with LR (Fig. 2). Similarly, platelet counts were reduced by hemorrhage and resuscitation, with greater reduction shown after Hextend resuscitation (Fig. 2)

There were no significant changes in coagulation factors in the control group (Table 2). After hemorrhage and LR resuscitation, factors II, V, VII, VIII, IX, X, XI, XII, and XIII decreased to 40% to 60% of corresponding baseline values. Rebound increases were observed in factors VIII, IX, X, and XII at 6 hours after LR resuscitation (Table 2). Even greater reductions were observed after hemorrhage and Hextend resuscitation, particularly with regard to factors VIII, XI, and XII compared with the LR group. Factors VIII level fell the most to 14% ± 2% of baseline values of all the factors measured and did not rebound at 6 hours (Table 2).

Coagulation Functional Assessments

No significant changes were observed in any TEG variables in the control group over time. The initial clotting time (*R* time) was shortened by hemorrhage in both hemorrhaged groups and returned to prehemorrhage levels immediately after Hextend resuscitation and by 3 hours after LR resuscitation (Fig. 3). Clotting rapidity (α) was accelerated by hemorrhage in both groups and returned to near-prehemorrhage level immediately after LR resuscitation and by 6 hours after Hextend resuscitation (Fig. 3). Clot strength (MA) did not change with hemorrhage but decreased after resuscitation in both groups and returned to prehemorrhage levels by 3 hours after LR resuscitation (Fig. 4). A greater and prolonged decrease of MA was observed after Hextend resuscitation throughout the study (Fig. 4). Overall clotting capacity (CI) did not change with hemorrhage in either group, was decreased by LR resuscitation, but returned to baseline by 3 hours, whereas CI after Hextend resuscitation was decreased further and did not return to prehemorrhage levels at the end of the study (Fig. 4). No significant changes in LY_{30} or LY_{60} (fibrinolysis) were observed in any group during the study period (data not shown).

DISCUSSION

Different crystalloids and colloids have been routinely used to treat hypovolemia in patients. However, debates continue over the selection of ideal resuscitative fluids.[3,27–32] As Hextend is the recommended fluid for battlefield resuscitation by the military, controversy remains whether it is the best fluid. In our effort of searching for optimum resuscitation fluids, we comprehensively investigated the effects of Hextend and LR in an animal model with traumatic hemorrhage in this study. Following femur fracture and 60% blood loss, MAP was decreased to 50%

TABLE 1. Changes in Blood Gas and Chemistry

	Baseline	Hemorrhage	Resuscitation	3 h	6 h
Lactate, mM					
Control	2.2 ± 0.3	2.2 ± 0.2	1.9 ± 0.2	1.8 ± 0.2	1.8 ± 0.2
LR	2.2 ± 0.2	10.2 ± 0.8*	9.0 ± 0.7*	4.1 ± 0.7*	5.8 ± 1.9*
Hextend	2.1 ± 0.2	9.9 ± 1.1*	10.4 ± 1.2*	3.8 ± 0.6*	2.9 ± 0.7†
Base excess, mM					
Control	5.3 ± 0.7	6.2 ± 0.6	6.6 ± 0.7	7.0 ± 0.6	6.2 ± 0.5
LR	5.8 ± 0.5	−3.7 ± 0.6*	−0.4 ± 0.8*	4.3 ± 0.9*	3.1 ± 1.8*
Hextend	5.3 ± 0.9	−3.3 ± 1.2*	−1.9 ± 1.3*	6.5 ± 0.9	6.8 ± 1.1
Hematocrit, %					
Control	28 ± 1	28 ± 1	28 ± 1	25 ± 3	28 ± 1
LR	28 ± 1	27 ± 2	13 ± 1*	15 ± 1*	15 ± 1*
Hextend	28 ± 1	26 ± 1	8 ± 0*†	9 ± 1*†	9 ± 1*†
Red blood cell, $10^6/\mu L$					
Control	5.7 ± 0.2	5.6 ± 0.2	5.6 ± 0.2	5.7 ± 0.2	5.6 ± 0.2
LR	5.5 ± 0.2	5.2 ± 0.2	2.4 ± 0.1*	3.0 ± 0.2*	3.0 ± 0.1*
Hextend	5.4 ± 0.2	5.0 ± 0.2	1.6 ± 0.1*†	1.7 ± 0.1*†	1.8 ± 0.1*†
Hemoglobin, g/dL					
Control	9.6 ± 0.2	9.4 ± 0.2	9.5 ± 0.2	9.5 ± 0.2	9.6 ± 0.2
LR	9.3 ± 0.3	8.9 ± 0.5	4.1 ± 0.3*	5.2 ± 0.4*	5.1 ± 0.2*
Hextend	9.3 ± 0.2	8.6 ± 0.3	2.7 ±± 0.2*†	2.9 ± 0.2*†	3.2 ± 0.2*†
Total protein, g/L					
Control	5.4 ± 0.1	5.3 ± 0.1	5.3 ± 0.2	5.3 ± 0.2	5.1 ± 0.2
LR	5.1 ± 0.2	3.9 ± 0.2	2.4± 0.1*	3.2 ± 0.1*	3.1± 0.1*
Hextend	5.4 ± 0.1	4.2 ± 0.1	<2.0	2.0 ± 0.1*†	2.2 ± 0.1*†
Amylase, U/L					
Control	1,747 ± 219	1,708 ± 186	1,708 ± 194	1,822 ± 193	1,759 ± 173
LR	2,091 ± 225	1,884 ± 311	1,302 ± 242*	1,343 ± 220*	1,198 ± 93*
Hextend	1,796 ± 447	1,390 ± 515	775 ± 310*†	1,311 ± 466†	1,551 ± 568†

*$p < 0.05$ compared with the corresponding Day 1 baseline values. n = 7 per group.
†$p < 0.05$ LR compared with Hextend. n = 7 per group.

of prehemorrhage levels in this study. A 1:1 replacement of shed blood volume with Hextend (42 mL/kg) was infused, which returned MAP to near-prehemorrhage levels, whereas three times volume of LR solution (118 mL/kg) was infused to reach the same MAP. Base excess and lactate returned to prehemorrhage levels at end of the study after Hextend infusion but not with LR resuscitation. Thus, Hextend seems to be effective for expanding volume and maintaining acid-base balance. This advantage, however, comes with undesired effects on coagulation.

Coagulation was adversely affected by hemorrhage and resuscitation with LR or Hextend in this study, with more severe impairment shown from Hextend resuscitation. Furthermore, clot strength and clotting index returned to prehemorrhage levels by 3 hours after LR resuscitation, but no recoveries were observed after Hextend resuscitation during the 6-hour period of the study. Thus, to reach the same resuscitation goal, Hextend resuscitation resulted in more severe and long-lasting impairment on coagulation.

The greater coagulation effects observed in the Hextend group seem to be related to its volume expansion properties, although as a hetastarch product, it was not considered to have significant effects on coagulation because it contains calcium. Specifically, platelet counts dropped to 50% and 20% of baseline values with LR and Hextend, respectively. Plasma fibrinogen concentration, however, was similarly decreased upon the completion of LR or Hextend resuscitation. Afterward, an increase of fibrinogen from LR resuscitation was observed during the 6-hour observation, but no recovery occurred with Hextend resuscitation. The increase of fibrinogen after LR resuscitation was associated with the return of clot strength and overall clotting index. Since fibrinogen and platelet are primary contributors to clot strength and platelet counts remained depleted during the 6-hour study, the return of clot strength in LR group was likely caused by the increase of fibrinogen availability. This notion was further supported by the results from the Hextend group since the lower levels in platelet and fibrinogen amounts were associated with no recovery of coagulation function. Consistently, Nielsen[33] reported that the impairment of Hextend on clot strength (MA) was recovered by addition of fibrinogen in vitro. Put together, these data suggested that an increase in fibrinogen, such as from endogenous production or exogenous administration, might likely recover clot strength impaired by Hextend resuscitation.

Similar to changes of platelet count, larger reductions of coagulation factors were observed with Hextend resuscitation

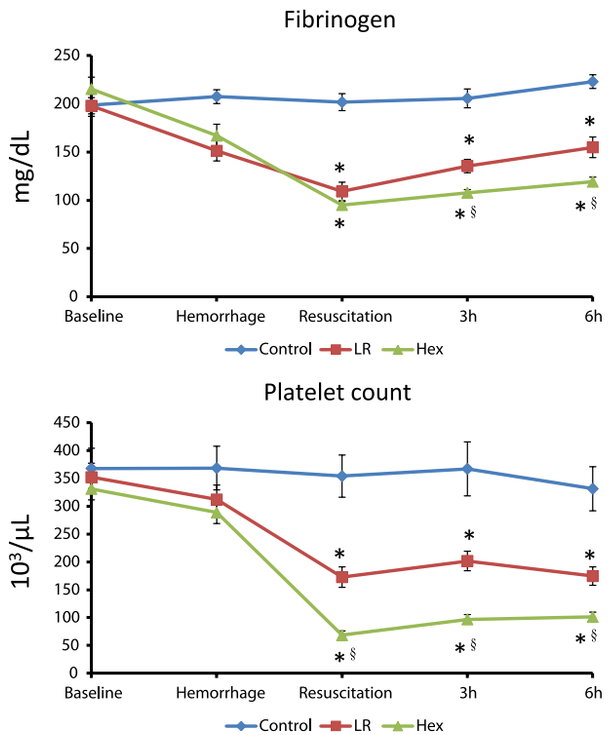

Figure 2. Changes in fibrinogen concentration and platelet counts after femur fracture, hemorrhage, and LR solution or Hextend resuscitation in pigs. Data are presented as mean ± SEM for 7 animals per group. *$p < 0.05$ compared with the corresponding baseline values. §$p < 0.05$ LR group compared with the Hextend group.

in this study. Factors II, V, VII, VIII, IX, XI, XII, and XIII were reduced to 15% to 50% of prehemorrhage levels by Hextend resuscitation, compared with 40% to 60% by LR resuscitation. Among these factors, factor VIII with Hextend resuscitation had the largest drop of 85%. Similar findings were observed in studies from a rabbit model and in vitro.[34–35] The larger drop of factor VIII is considered to be related to the inhibition of Hextend on factor VIII release from endothelial layer or Hextend binding to coagulation factors by colloid macromolecules.[35–36] It is not clear why the other coagulation factors were not similarly affected.

As mentioned, with the unique logistical and tactical constraints of the far-forward military environment, the Tactic Combat Casualty Care (TCCC) guidelines recommend up to a liter of Hextend for resuscitation of combat casualties owing to its high efficacy in volume expansion.[8] In this severe hemorrhage (60% blood loss, shed blood volume 42 mL/kg) animal model, animals received a 1:1 resuscitation with Hextend–to–shed blood volume, which is often used in experimental studies. [37] This is approximately three times the dose of Hextend recommended by the TCCC committee (14 mL/kg) and was used not to test the TCCC recommendation but to match the blood pressure response to LR resuscitation. Our study adds new information on Hextend resuscitation and prolonged effects on coagulation and may lead to reevaluation of current TCCC guideline of giving up to a liter of Hextend as an initial fluid therapy. In addition, the prolonged coagulation impairment observed in this study cautions the use of Hextend. With a volume distribution of 72-mL blood per kilogram of body weight, Hextend is primarily confined in the vascular pool.[38] A half-life of 38 hours[38] indicates that Hextend is metabolized slowly by amylase, despite a fast increase in blood amylase level observed in this study. The elevation of amylase was likely resulted from a feedback mechanism induced by Hextend infusion. The slow metabolism of Hextend contributes to the prolonged hemodilution observed in this study. Since the metabolic half-life of hydroxyethyl starch depends on the percentage of HES substitution, a lower mean MW starch with less substitution, such as Voluven, may minimize the long-lasting adverse effects of Hextend on coagulation. This notion is supported by a

TABLE 2. Changes of Coagulation Factors Following Femur Injury and Hemorrhage With LR or Hextend Resuscitation in Pigs

	Hemorrhage and Resuscitation	6 h
Factor II		
Control	98 ± 3	91 ± 5
LR	43 ± 4*	53 ± 4*
Hextend	33 ± 4*	39 ± 5*
Factor V		
Control	99 ± 3	95 ± 2
LR	46 ± 6*	47 ± 5*
Hextend	32 ± 1*	29 ± 4*
Factor VII		
Control	101 ± 3	94 ± 4
LR	43 ± 5*	40 ± 5*
Hextend	35 ± 3*	35 ± 5*
Factor VIII		
Control	100 ± 3	102 ± 5
LR	50 ± 6*	75 ± 10*
Hextend	14 ± 2*†	16 ± 2*†
Factor IX		
Control	103 ± 2	101 ± 1
LR	44 ± 3*	60 ± 5*
Hextend	28 ± 4*	41 ± 6*
Factor X		
Control	103 ± 3	95 ± 5
LR	38 ± 7*	47 ± 7*
Hextend	33 ± 2*	47 ± 9*
Factor XI		
Control	109 ± 7	99 ± 6
LR	39 ± 6*	46 ± 3*
Hextend	27 ± 2*†	29 ± 2*†
Factor XII		
Control	96 ± 2	97 ± 2
LR	43 ± 7*	64 ± 5*
Hextend	25 ± 2*†	37 ± 2*†
Factor XIII		
Control	101 ± 2	104 ± 3
LR	62 ± 5*	69 ± 5*
Hextend	48 ± 4*	49 ± 3*

Data are expressed as % of corresponding baseline values in each group (n = 7).
*$p < 0.05$ compared with the corresponding baseline values.
†$p < 0.05$ LR versus Hextend.

clinical trial comparing the efficacy of Voluven (HES 130/0.4) and hetastarch (HES 670/0.75) in 100 patients who needed intravascular volume replacement during major orthopedic surgery.[7] The authors reported that the two colloid fluids were equally effective in plasma volume expansion but Voluven has a lesser effect on coagulation.[13] However, other clinical trials showed comparable results of blood loss in patients resuscitated with HES 130/0.4 or HES 200/0.5 during cardiac surgery.[14,39] Although doses of 6% HES 130/0.4 as high as 70 mg/kg can be used in critically ill head trauma patients,[40] future clinical and animal studies are warranted to confirm the effects of low MW starch with less substitution. Since the military makes small volume resuscitation without the use of blood products appealing, there is interest in investigating a modification of colloids as an adequate strategy rather than abandoning colloid resuscitation all together.

In this study, a 1:1 replacement of shed blood volume with Hextend was infused, which returned MAP to near-prehemorrhage levels. If hypotensive resuscitation proves beneficial following traumatic injury, different criteria, such as base deficit and lactate levels, should be used to represent tissue perfusion for earlier volume resuscitation. For the military, blood pressure measurements are not available in the prehospital setting, and so, presence of a radial pulse and mental status have been used to assess volume resuscitation. With the development of point-of-care noninvasive or minimally invasive base deficit and lactate sensors as well as tissue oxygenation sensors, we believe that such devices will improve resuscitation to keep the patient from going into shock and the pathophysiology that develops. This need has received much attention in recent years as the identification of the acute coagulopathy of trauma and its association with high mortality rates have amplified the need to prevent development of shock.

In summary, we performed a comprehensive investigation of the effects of Hextend and LR resuscitation on hemodynamic and coagulation during a 6-hour period in an animal model with traumatic injury and severe hemorrhage. With limited fluid resuscitation, Hextend was effective in restoring hemodynamic and acid-base status with a third of the volume of LR solution. The advantage of Hextend, however, was associated with undesired effects on coagulation. The long-lasting adverse effect on coagulation from Hextend was caused by more severe and sustained hemodilution, suggesting that doses of Hextend need to be limited as with the older starch products. Furthermore, when Hextend is used for patients with massive blood loss, coagulation changes need to be closely monitored for timely interventions.

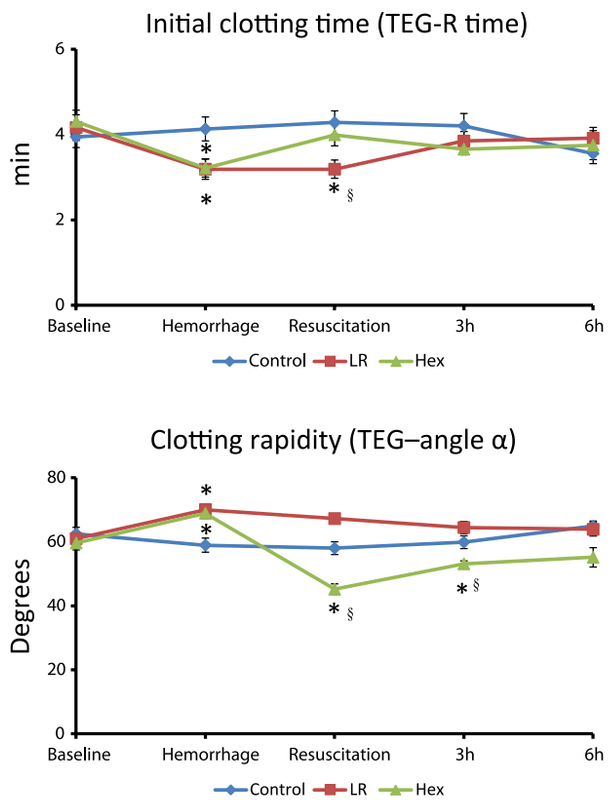

Figure 3. Changes in clotting initiation time (*R*) and clotting rapidity (α) from TEG measurements after femur fracture, hemorrhage, and LR solution or Hextend resuscitation in pigs. Data are presented as mean ± SEM for 7 animals per group. *$p < 0.05$ compared with the corresponding baseline values. §$p < 0.05$ LR group compared with the Hextend group.

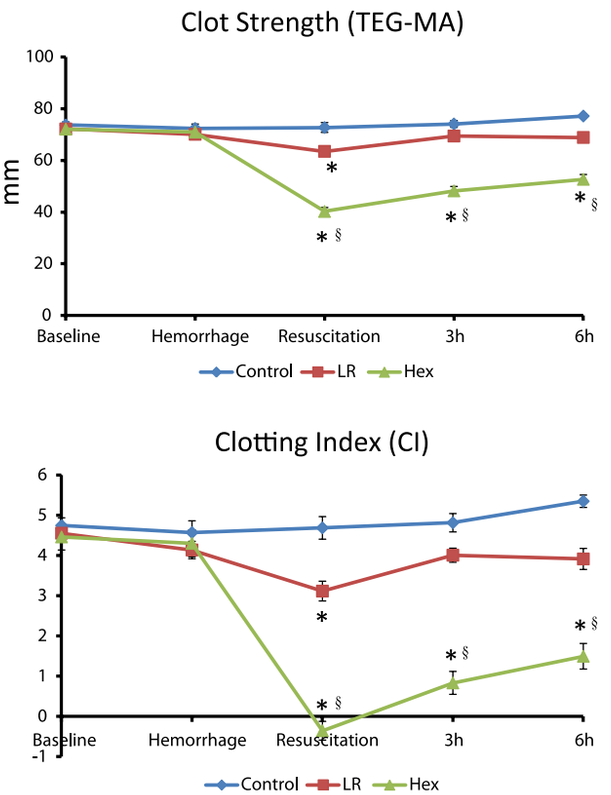

Figure 4. Changes in clot strength (MA) and clotting index from TEG measurements after femur fracture, hemorrhage, and LR solution or Hextend resuscitation in pigs. Data are presented as mean ± SEM for 7 animals per group. *$p < 0.05$ compared with the corresponding baseline values. §$p < 0.05$ LR group compared with the Hextend group.

AUTHORSHIP

W.Z.M. designed and performed the study, analyzed the data with assistance from statistician Mr. John Jones, and wrote the article. M.A.D. designed the study and edited the article. L.B. edited the article.

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DISCLOSURE

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DISCUSSION

Dr. David B. Hoyt (Chicago, Illinois): The selection of initial resuscitation is impacted by many considerations. The efficiency of perfusion restoration, that is the net amount of volume to restore to a perfusion target, the effect on the inflammatory response, and now, today, the effects on coagulation all have to be considered.

Currently, Hextend is the fluid that is selected by the Combat Casualty Care Committee due to the fact that at the time, over 10 years ago, hypertonic saline, which was also considered, was not FDA approved.

The utility of the lower weight was an important criteria relative to carrying Lactated Ringer's and at the end of the day I think tipped the balance toward Hextend.

Now, detailed evaluations of affecting clotting using animal models that duplicate severe injury are few in number. This study is one of the first to objectively look at the effects on coagulation, comparing Hextend to Lactated Ringer's.

Particularly concerning are the effects of Hextend on fibrinogen and platelets. These data suggest that Lactated Ringer's leads to a strong clot formation and would, therefore, appear to be a superior initial resuscitation fluid. The logistic disadvantage now has to be reconsidered. A few questions for the authors.

First, will data like this be successful in causing re-evaluation of the current Combat Casualty Care Guideline, which was essentially reaffirmed from the original one in 2000, and that is of giving up to a liter of Hextend as an initial fluid therapy?

Secondly, do you think strategies that would evolve or modify colloids such as volvulin is an adequate strategy rather than abandoning colloid resuscitation altogether if it has the same impact on coagulation?

Third, should alternative hypertonic crystalloid solutions be studied for their logistic advantage?

And, fourth, should we be using different criteria for early volume resuscitation endpoints if hypotensive resuscitation proves beneficial following an appropriate clinical trial?

The authors are to be congratulated. This is exactly the kind of study that needs to continue today to reassess the importance of previous recommendations. And when newer data such as this affecting the coagulation profile become apparent, we need to consider how this will affect patient care.

Dr. Charles E. Lucas (Detroit, Michigan): The great Frannie Moore in 1955 showed that the addition of colloid caused a coagulopathy. He didn't know what the reason was.

Myself and my white-haired partner in the 1980s demonstrated in random studies that albumin in man and all sorts of colloids in animal cause a coagulopathy by driving the coagulation factors out of the plasma into the interstitial space where they can be measured in the lymphatics.

This was a dose response-type phenomenon, a little bit of Hespan gives a little bit of coagulopathy; a lot gives a lot. I compliment you on your beautiful study and I'm wondering what you are doing to get the military to stop poisoning our troops with colloid.

Dr. Hasan B. Alam (Ann Arbor, Michigan): I have a similar kind of question. As you probably know a conference was recently held at the NIH that the FDA had put together, with DoD and NHLBI involvement, looking at hydroxyethyl starch solutions.

And for two days last week we sat and listened to study after study after study showing the same thing. In preclinical and clinical studies, large molecule and small molecule hetastarches, septic and hemorrhagic shock models, it seems to cause dose-dependent toxicity. Resuscitation with these solutions is associated with coagulopathy, inflammation, and end organ damage such as acute kidney injury, and worse survival. I suspect that the FDA is going to deliberate and either come out with the black box indication or put a qualifier that it should not be used in hemorrhagic shock patients.

So my question is that in the face of all these negative data, is there really any role for hydroxyethyl starch solutions for resuscitating patients in hemorrhagic shock? And what should the DoD do? I think the data are fairly compelling that the use of these fluids for the treatment of hemorrhagic shock should be abandoned.

Dr. Martin Schreiber (Portland, Oregon): Jun, nice study. I am really confused, though, about the methods. The abstract says that you gave Hextend 1:1 with blood loss and you gave LR 3:1, but those aren't consistent with the ratios in your abstract.

And in your presentation you said that you gave the equivalent amount of LR to get the same blood pressure rise that you got with Hextend so I'm not sure how you did that. So could you please clarify the methods there?

The second point is that this volume of Hextend, 1600 ccs, is really a huge volume, and especially when you consider the size of the pigs. I assume they are 30–40 kilogram pigs.

So you're giving a huge dose of Hextend. The maximal dose as described by Dr. Frank Butler, standing at the other microphone, is one liter, so why would you give such a huge dose of Hextend?

Finally, I just want to make a comment to Dr. Charlie Lucas who talked about poisoning war fighters, the truth of the matter is despite the Herculean efforts by Dr. Butler, the vast majority of war fighters get normal saline in the field when they are injured.

Dr. Frank Butler (Pensacola, Florida): I think this is a tremendous contribution to our knowledge base and really applaud the effort that you all have displayed here.

A couple of points. I think you have shown us that both Hextend given in a dose one-third that of the Lactated Ringer's required has produced 100% survival in your model for six hours with 60% total blood loss. That's interesting.

I think it will be even more interesting if you undertake an uncontrolled hemorrhage model. We think we have a pretty good handle on controlled hemorrhage but if we can look at the same question in the uncontrolled hemorrhage model that would be very valuable.

Dr. Wenjun Martini (San Antonio, Texas): Let me start with our thanks to AAST, Dr. Livingston and Dr. Leppaniemi

for the privilege, and Dr. Hoyt and others for their encouraging comments and wonderful questions.

In response to Dr. Hoyt's first question: with the data from this study, should we reevaluate the current combat casualty guidelines? Hextend was recommended for use in the military by the Committee on Tactical Combat Casualty Care (CoTCCC) over 10 years ago. CoTCCC guideline limited Hextend to 1L in order to reduce risk of bleeding complications. Literature is beginning to accumulate showing Hextend at doses greater than CoTCCC limit can affect coagulation. However, at present there is still no FDA approved small volume fluid proven to be better than Hextend for battlefield or pre-hospital use. Thus, we are still searching for a better fluid, as well as investigating what was put in the guideline, as shown in this study which comprehensively investigated the effects of Hextend on coagulation and physiology.

Fresh frozen plasma (FFP) has been put forward in the last discussion by CoTCCC. FFP is FDA approved but logistically of limited availability on the battlefield. Progress is being made with freeze dried plasma and is just being introduced to some limited units for forward use.

The second question is about Voluven. The effects of colloid fluids relate to the degrees of hydroxyl group substitution and molecular weights. Compared to Hextend, Voluven has a less degree of substitution and lower molecular weight. So in theory we can speculate that it could have less adverse effects on coagulation. And the company states that it can be used at doses as high as 50 ml/kg without adverse effects on bleeding. So we will see if it is better. But the published literature is not complete. Some studies show improved effects while others show the same. So we don't know the answer until we do the study.

Next question is the effects of hypertonic crystalloid solutions. Yes, hypertonic saline also has logistic advantages. Since the 7.5% solution extensively studied has not been approved by FDA, we haven't done a comparative study, thus we don't know whether it is better than Hextend.

The last question from Dr Hoyt is about criteria for early fluid resuscitation. If hypotensive resuscitation proves beneficial following traumatic injury, different criteria, such as base deficit and lactate levels, should be used to represent tissue perfusion for earlier volume resuscitation. For the military, blood pressure measurements are not available pre-hospital and so, presence of a radial pulse and mental status have been used to assess volume resuscitation. With development of point of care non-invasive or minimally invasive base deficit and lactate sensors and tissue oxygenation sensors, we believe that such devices will improve resuscitation to keep the patient from going into shock and the pathophysiology that develops. This need has received much attention in recent years as the identification of the acute coagulopathy of trauma and its association with high mortality rates has amplified the need to prevent development of shock.

In response to Dr. Lucas's comment, Hextend was selected for its logistic advantages and was FDA approved. A recent prospective study from 2009 to 2011, (J Trauma Acute Care Surg. 2012, 73(2)S38–42) showed that the uses of normal saline, lactated Ringer's, and colloids (Hextend) in battlefield care is 73%, 17% and 8%, respectively. Thus, normal saline is still the most commonly used. We are still seeking an improved fluid that is suitable for use in the battlefield.

In response to Dr Alam's question, as mentioned, at present there is no small volume fluid for battlefield use proven to be better than Hextend that is FDA approved. Hextend was put in the guideline as the most logical choice at the time. We continue to study its effects and explore other options.

To respond Dr. Schreiber's first question, I mentioned in the presentation that Hextend and LR were used to reach equivalent MAP for comparison. In the Hextend group, Hextend was infused as 1:1 volume replacement. The 60% hemorrhage bled out 42ml/kg blood, so 42ml/kg Hextend was infused. In the LR group, LR was infused to reach the same MAP as in the Hextend group, which turned out that 118 ml/kg of LR was needed to reach the same MAP.

Second question is about the volume of Hextend used. We designed the study as 1:1 volume replacement with Hextend. This is a severe hemorrhage model, which turned out to be about three times of the dose recommended in the guideline. The selection of this volume was based on our study aims and design. Thus, we are aware that the current results should be interpreted with caution in extrapolating back to the CoTCCC guidelines.

In response to Dr Butler's suggestion, yes, as a follow-up, we will pursue with uncontrolled hemorrhage model.

Thank you all, again.